REMARKS

The Claims

Claims 35-37, 42-51, 53, and 60-80 are currently pending in this application. Claims 35, 53, 61, and 65-80 have been amended. For the purpose of expediting prosecution of the application, applicants have cancelled claims 1-15, 22-26, 31-34, 38-41, 52, and 54-60 and reserve the right to file a continuation or divisional application claiming the subject matter of the cancelled claims which continuation or divisional application claims priority to the present application. Furthermore, claims 16-21 and 27-30 have been withdrawn as they are directed to non-elected groups and applicants reserve the right to file a continuation or divisional application claiming the subject matter and claiming priority from the present application. Claims 43-51 have been allowed. No new matter is being hereby introduced.

Support for the amendments made to claims 35 and 80, where the modified pneumolysin polypeptide has one or more than one amino acid substitution, may be found at page 34, lines 19-34 of the instant specification.

Claim 53 has been amended into dependent form, and adds the subject matter of claim 60, which has been cancelled.

As claim 60 has been cancelled and incorporated into claim 53, claim 61 now depends from claim 53 instead of claim 60.

Applicants respectfully submit that these amendments do not constitute new matter and respectfully request entry thereof.

Objection Maintained

8) Applicants note that the informal drawings filed in this application have been accepted. Drawings will be submitted upon allowance of this application.

Rejections Maintained

Claim Rejections – 35 U.S.C. §112, first paragraph- Enablement

9) Claims 35-37, 42, 53, and 60-80 stand rejected under 35 U.S.C. 112, first paragraph, on the grounds that the specification is non-enabled with regard to the scope. It is the Examiner's position that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Applicants respectfully disagree with the Examiner's contention that the subject matter of claims 35, 53 and 80, and dependent claims therefrom, is non-enabled. Applicants believe that the wording of claims 35, 53 and 80 is correct and clear. The Examiner's understanding that the recitation of "at least one" in line 2 of claim 35 is correct in that at least two types of modified pneumolysin polypeptides are encompassed within the scope of the claim. Applicants are claiming a modified pneumolysin polypeptide having (i) more than one (1) amino acid substitution of SEQ ID NO:3 at a position selected from the group consisting of position 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257; and (ii) a modified pneumolysin polypeptide having only one amino acid substitution, where the substitution occurs at a position selected from the group consisting of positions: 61, 148, and 195.

The Examiner's suggestion of claiming a modified pneumolysin comprising an amino acid substitution at position 61, 148, or 195 alone or in combination with at least one amino acid substitution selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 is not completely correct (emphasis added). Rather, if the modified pneumolysin polypeptide has only one amino acid substitution, then it occurs at position 61, 148 or 195. Otherwise, the modified pneumolysin polypeptide has two or more amino acid substitutions at positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255 or 257.

The modified pneumolysin polypeptides as claimed have the recited three functions. Applicants respectfully disagree with the Examiner's contention that "at least

one of the recited functions in these single mutants which are currently encompassed in the scope of the claim is contrary to what is being recited." The Examiner specifically points to the instant specification at pages 25 and 26, which recites that amino acid residues which may be substituted but which alone do not reduce hemolytic activity include those at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 127, 128, 172, 189, 239, 255, and 257. As previously discussed, the modified pneumolysin polypeptide having only one amino acid substitution is selected from the group consisting of positions 61, 148, and 195, as claimed in Claims 35 and 80. The other type of modified pneumolysin polypeptide has more than one amino acid substitution selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257. Therefore, applicants assert that as the instant specification recites at pages 25 and 26, those modified pneumolysin polypeptides having the recited functions do not include single amino acid substitutions at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255, and 257. Non-limiting examples of the modified pneumolysin polypeptide of the instant invention include a modified pneumolysin polypeptides having preferred substitutions of: (1) Ile33Thr, Ile46Thr, Leu83Ser, Ser239Arg, and Asp257Gly; (2) Lys17Arg, Lys18Asn, Ser61Pro, Asn66Tyr, and Ile101Thr; (3) Asp41Gly, Thr172Ala, Phe195Ile, and Lys255Gly; (4) Ile33Thr, Ile46Thr, Leu83Ser, Ser239Arg, and Asp257Gly; and (5) Val45Ala, Asp102Gly, Gln189, Arg, Phe195Val (see Table 5A). The specification provides 5 peptides having four (4) or five (5) substitutions among the recited amino acids which all result in modified pneumolysins having the required 3 functions of solubility, capability to elicit antibodies cross reactive with wild type pneumolysin, and attenuated hemolytic activity. In view of the disclosure of these peptides, one skilled in the art would be able to make other pneumolysins which encompass other combinations of these substitutions and which would also have the required functions without undue experimentation. Thus, the instant specification supports the claimed invention.

The Examiner contends Lazar, et al. and Hansen, et al. demonstrate unpredictability with regard to the structure-function association in a polypeptide. In

particular, the Examiner contends that since retention of a single function upon one conservative amino acid substitution is unpredictable, retention of more than one function upon one or more conservative or non-conservative amino acid substitutions would not have been predictable. The Examiner further contends that the issue at hand is whether applicants enable the modified pneumolysin polypeptide of claims 65-67 and 77 without undue experimentation in view of the unpredicability of Lazar, et al. and Hansen, et al.

It is well established that applicants do not need to exemplify every claimed embodiment. *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970). Based on the data presented, one skilled in the art would reasonably expect to effectively substitute amino acids with the claimed amino acids at specified positions. The specification clearly enumerates specific parameters that the modified pneumolysin polypeptides having the claimed amino acid substitutions must have, such as, solubility, capability to elicit antibodies cross-reactive with wild-type pneumolysin, and attenuated hemolytic activity. One skilled in the art would understand that amino acid substitutions with amino acids having similar properties are generally accepted to be conservative. In fact, as the Examiner has pointed out, Lazar, et al. "did **not** expect that a mutation of Leu to Ile (which have similar sizes and polarities) would cause such a strong effect" upon mutating functionally equivalent amino acids. This suggests that the accepted understanding in the art is that conservative amino acids behave similarly. Therefore, the claims are commensurate with the specification since the claims are drawn specifically to the invention as set forth in the specification.

In *In re Angstadt*, 537 F.2d 498 (C.C.P.A. 1976), the court considered as unpredictable the subject matter of the application in issue, catalytic processes. The court also acknowledged that the scope of enablement varies inversely with the degree of unpredictability involved. The court even stated that "[a]ppellants have apparently not disclosed every catalyst which will work; they have apparently not disclosed every catalyst which will not work. *Id.* at 502. The court decided that "appellants are not required to disclose every species encompassed by their claims even in an unpredictable art such as the present record presents, each case must be determined on its own facts."

Id. at 503. The present case, like the case in *Angstadt*, provides the public with a reasonable and finite list of possible variables to choose from in preparing the object of the invention. Neither of the processes, *Angstadt's* and present applicants', are complicated and no special equipment or reaction conditions are required. Thus, here, like in the case of *Angstadt*, there is no basis for concluding that persons skilled in this art, armed with the specification and its examples, would not easily be able to determine which modified pneumolysin polypeptides are refolded and have attenuated activity and which do not.

Like the applicants in *Angstadt*, working examples are provided in the present specification. "If one skilled in this art wished to make and use a [modified pneumolysin polypeptide that is soluble, elicits antibodies cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity] other than those disclosed, [he or she] would merely read [applicants'] specification for directions how to make [a modified pneumolysin polypeptide,] and could then determine whether [a modified pneumolysin polypeptide that is soluble, elicits antibodies cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity is], in fact, formed." See, *In re Angstadt* at 503. Since applicants have provided a simple method for producing the claimed modified pneumolysin polypeptide, the experimentation required to determine additional modified pneumolysin polypeptides would not be undue and certainly would not require ingenuity beyond that to be expected of one of ordinary skill in the art.

In addition to providing amino acids appropriate for substituting at particular positions, applicants also describe certain structure function relationships which are associated with solubility, capability of eliciting antibodies that are cross-reactive with wild-type pneumolysin, and reduced hemolytic activity. These structure function parameters also enable one skilled in the art to make and use the claimed invention. Applicants describe assays suitable for testing the hemolytic activity of the modified pneumolysin polypeptides. In addition, those skilled in the art, using routine protocols could readily determine whether the modified pneumolysin polypeptides have the three recited functions. None of these determinations would require undue experimentation

and thus contrary to the assertions of the Examiner, section §112, first paragraph is satisfied. Moreover, though applicants examples and identification of specific amino acid positions which may be substituted to achieve the claimed modified pneumolysins, applicants have provided sufficient information to those skilled in the art to make other modified pneumolysins based on the disclosed structural and functional information.

However, in order to address the Examiner's concerns, claims 35, 53, 65-68, and 80 have been amended for clarification. Applicants respectfully submit that the rejection to claims 35-37, 42, 53, and 60-80 under 35 U.S.C. §112, first paragraph has been overcome and respectfully request reconsideration and withdrawal of this rejection to the claims on this ground.

Claim Rejections – 35 U.S.C. §112, second paragraph

25) Claims 35-37, 42, and 60-80 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants respectfully traverse the rejection.

(a) In particular, the Examiner finds claim 35 vague, indefinite, confusing and internally inconsistent in the recitation: 'comprising substituting at least one amino acid...selected from the group consisting of...17, 18, 33, 41, 45, 46,...66, 83, 101, 102, 128,...189,...239, 255, and 257...wherein...possesses only one substitution...positions 61, 148 and 195.' Applicants assert that the Examiner has misinterpreted claim 35, in that the claim recites or encompasses only one amino acid substitution at position 61, 148, or 195, and more than one amino acid substitution at positions selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, including positions 61, 148, and 195. Contrary to the Examiner's interpretation, when the modified pneumolysin polypeptide has more than one amino acid substitution, the substitutions do not require that at least one be from position 61, 148, or 195. Therefore, the Examiner's proposed amendment of claim 35 (Office Action dated May 6, 2003: pg. 13, section 28) does not correctly reflect the

instant invention. However, claim 35 has been amended for clarification in order to address the Examiner's concerns.

(b) Claim 80 is also found to be indefinite for analogous reasons to those applied to claim 35. The Examiner rejects claim 80 because the metes and bounds are allegedly incomprehensible. Applicants respectfully disagree with this rejection for the reasons set forth in section (a) above. However, claim 80 has been amended for clarification in order to address the Examiner's concerns.

(c) The Examiner has further rejected to for claim 35 for being confusing and unclear as to antecedence basis for the recitation: "said modified pneumolysin" in lines 6 and 7 of the claim. Applicants respectfully disagree with this rejection. However, in order to address the Examiner's concerns, applicants have amended claim 35.

(d) Claim 65 has been rejected to for the recitation: "The polypeptide according to claim 35...said modified pneumolysin polypeptide...." Applicants have amended claim 65, as suggested by the Examiner, by replacing the recitation with - -The modified polypeptide according to claim 35, wherein said one amino acid substitution at position 61 is a proline or hydroxyproline substitution- -.

(e) Analogous criticism to that of claim 65 has been applied to claims 66-68. Applicants have amended claims 66-68 similarly to the amendments made to claim 65 as indicated above in section (d) in order to address the Examiner's concerns.

(f) Claim 80 has been rejected to for being vague, indefinite, and confusing in the recitation "substituting at least one amino acid sequence having SEQ ID NO: 3," because the Examiner contends that it is unclear how and with what the entire sequence of SEQ ID NO: 3 is substituted. Applicants have amended claim 80 to recite - - substituting at least one amino acid of SEQ ID NO: 3- - in order to address the Examiner's concerns.

(g) Claim 80 has been further rejected to for being indefinite, confusing, and/or improper in the recitation: "substitution is at a position selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257....wherein the amino acid substitution at position

127...wherein the amino acid substitution is at position 172...." Applicants have amended claim 80 by deleting reference to amino acid substitutions at positions 127 and 172.

(h) The Examiner has also found claim 80 to be indefinite, confusing and inconsistent with the disclosure because the claim allegedly encompasses numerous modified pneumolysin polypeptides including those having one amino acid substitution at a recited position which is other than position 61, 148, and 195, where the modified polypeptide has the recited functions. The Examiner points to the sentence bridging pages 25 and 26 of the instant specification to indicate that the claim improperly includes several modified polypeptides without one of the three recited and required functions. Applicants assert that the modified pneumolysin polypeptide having an amino acid substitution selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255, and 257 of SEQ ID NO: 3, has more than one amino acid substitution, while the modified pneumolysin polypeptide with only one amino acid has an amino acid substitution selected from the group consisting of positions 61, 148, and 195. Thus, the instant specification is in accordance with the claimed modified pneumolysin polypeptide.

With respect to an amino acid substitution at position 243, the Examiner points to pages 57 and 58 of the instant specification for allegedly disclosing a mutation at position 243 that results in an insoluble, aggregate-yielding modified pneumolysin polypeptide. However, applicants direct the Examiner's attention to Table 5B which merely shows mutants having single amino acid substitutions at positions 243, 286, and 446 and the combined substitutions at positions 243 and 446 that do not properly refold. Claim 80 does not include a modified pneumolysin polypeptide having substituted amino acids at position 243 alone. Applicants assert that the modified pneumolysin polypeptide having an amino acid substitution at position 243 must also have at least one other substitution at positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 255, and 257. Furthermore, claim 80 does not include a modified pneumolysin polypeptide

having a combination of amino acid substitutions at positions 243 and 446, as suggested by Table 5B and the description at pages 57 and 58.

(i) Claims 36-37, 42, and 60-79, which depend directly or indirectly from claim 35, stand rejected under 35 U.S.C. §112, second paragraph as being indefinite because of the alleged vagueness or indefiniteness identified in the base claim 35. Applicants respectfully disagree with this rejection in view of the arguments and amendments made of claim 35.

Therefore, applicants respectfully submit that these 35 U.S.C. §112, second paragraph rejections have been overcome and respectfully request reconsideration and withdrawal of these rejections to claims 35-37, 42, and 60-80.

Claim Rejections – 35 U.S.C. §102

26) Claim 53 stands rejected under 35 U.S.C. §102(b) as being anticipated by Lock, et al. (Microb. Pathogen. 21:71-83, 1996), or Lee, et al. (Vaccine 12:875-878, 1994), or Paton, et al. (Infect. Immun. 59:2297-2304, 1991), or Alexander, et al. (Infect. Immun. 62:5683-5688, 1994). Applicants respectfully disagree with the Examiner's rejection.

Lock, et al. report that clinical isolates of *Streptococcus pneumoniae* alone contain mutations and show reduced hemolytic activity and increased molecular weight compared to wild type pneumolysin. Lock, et al. also briefly mention without presenting data that antibodies raised against the wild type pneumolysin neutralizes the hemolytic activity of the mutated pneumolysin and vice versa.

Lee, et al. report that when non-toxic pneumolysin derivatives conjugated to *Streptococcus pneumoniae* type 19F polysaccarides are used to immunize mice mothers, the infant mice have higher levels of antibodies against the wild type pneumolysin when compared to the control infant mice. These pneumolysin derivatives are mutated by site-directed mutagenesis.

Paton, et al. report that two pneumolysoids, Pd-A and Pd-B, constructed by oligonucleotide-mediated, site-directed mutagenesis of the cloned pneumolysin gene, have reduced hemolytic activities compared to that of native pneumolysin. The

hemolytic activity of Pd-B was also found to be 5 times less than that of Pd-A. According to Paton, et al., both of the pneumolysoids are at least as protective as native pneumolysin when immunized in mice challenged with *S. pneumoniae* as determined by mice survival times. Anti-pneumolysin levels induced by the Pd-B – 19F PS conjugate when compared to multiple doses of unconjugated pneumolysin or pneumolysoid was modest.

Alexander, et al. report of a pneumolysin toxoid that is protective against at least nine serotypes of *S. pneumoniae*. Specifically, PdB, constructed by oligonucleotide-directed mutagenesis and having reduced hemolytic activity, was found to provide non-serotype-specific protection against *S. pneumoniae*.

Claim 53 as currently amended is directed to the modified pneumolysin polysaccharide of claim 35 that is soluble, elicits antibodies that are cross-reactive with wild-type pneumolysin, has attenuated hemolytic activity, is obtained by random mutation of a nucleic acid encoding a pneumolysin polypeptide, and where the modified pneumolysin polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide. Lock, et al. is not directed to a conjugated modified pneumolysin polypeptide. Lee, et al., Paton, et al. and Alexander, et al. all report use of a pneumolysoid, Pd-B, which has a tryptophan to phenylalanine amino acid substitution at position 433 (Trp433Phe) and additionally, Paton, et al. report use of a pneumolysin Pd-A, which has a cysteine to glycine at position 428 (Cys428Gly). The modified pneumolysin polypeptide claimed in the instant invention does not have amino acid substitutions at either position 428 or 433. Therefore, none of the cited references, Lock, et al., Lee, et al., Paton, et al., and Alexander, et al. anticipate the claimed invention as currently amended. Applicants respectfully request withdrawal and reconsideration of this §102 rejection in view of the above-presented arguments.

It is submitted, therefore, that claims 35-37, 42, and 60-80 are in condition for allowance. Reconsideration of the Examiner's rejections is respectfully requested. Allowance of claims 35-37, 42, and 60-80 at an early date is respectfully solicited.

AUTHORIZATION

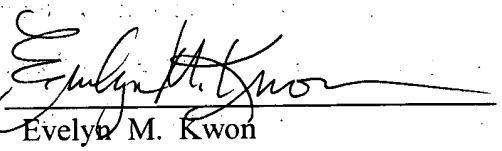
No additional fees are believed to be necessitated by this amendment. However, should this be an error, authorization is hereby given to charge Deposit Account No. 13-4500, Order No. 3842-4036US2 for any underpayment or to credit any overpayment.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Date: August 6, 2003

By: 

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